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REISSUE PATENT APPLICATION TRANSMITTAL	Attorney Docket No.	9491-050-27 REISSUE
	First Named Inventor	DENNIS J. SLAMON, ET AL.
	Original Patent Number	4,968,603
	Original Patent Issue Date	NOVEMBER 6, 1990
	Title	DETERMINATION OF STATUS IN NEOPLASTIC DISEASE

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OFFICE

APPLICATION FOR REISSUE OF: <input checked="" type="checkbox"/> Utility Patent <input type="checkbox"/> Design Patent <input type="checkbox"/> Plant Patent	
APPLICATION ELEMENTS	ADDRESS TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231
<p>1. <input checked="" type="checkbox"/> Fee Transmittal Form (Submit an original and a duplicate for fee processing)</p> <p>2. <input checked="" type="checkbox"/> Specification and claims</p> <p>3. <input type="checkbox"/> Drawing(s)</p> <p>4. <input checked="" type="checkbox"/> Reissue Oath or Declaration</p> <p>5. Original U.S. Patent <input checked="" type="checkbox"/> Offer to surrender original patent or <input type="checkbox"/> Ribbonded Original Patent Grant <input type="checkbox"/> Affidavit / Declaration of Loss</p> <p>6. Original U.S. Patent currently assigned? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No</p> <p>if yes, check applicable boxes</p> <p><input checked="" type="checkbox"/> Written Consent of all Assignees <input type="checkbox"/> Power of Attorney</p> <p><input checked="" type="checkbox"/> 37 C.F.R. §3.73(b) Statement</p>	<p>ACCOMPANYING APPLICATION PARTS</p> <p>7. <input type="checkbox"/> Transfer drawings from Patent File</p> <p>8. <input type="checkbox"/> Foreign Priority Claim (35 U.S.C. 119)</p> <p>9. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations</p> <p>10. <input type="checkbox"/> English Translation of Reissue Oath/Declaration</p> <p>11. <input type="checkbox"/> Small Entity Statement(s) <input type="checkbox"/> Statement filed in prior application. Status still proper and desired.</p> <p>12. <input checked="" type="checkbox"/> Preliminary Amendment</p> <p>13. <input checked="" type="checkbox"/> White Advance Serial No. Postcard</p> <p>14. <input checked="" type="checkbox"/> Other: ASSENT OF ASSIGNEE</p>
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REISSUE APPLICATION FEE TRANSMITTAL FORM					Docket Number 9491-050-27 REISSUE	
Claims as filed - Part 1						
Claims in Patent	For	Number Filed in Reissue Application	Number Extra	Rate		Fee
22	Total Claims	25	3	x	\$18 =	\$54.00
2	Independent	6	4	x	\$80 =	\$320.00
Basic Fee (37 CFR 1.16(h))						\$710.00
Total of above calculations						\$1,084.00
<input type="checkbox"/> Reduction by 50% for filing by small entity						\$0.00
Total Filing Fee						\$1,084.00

Claims as Amended - Part 2						
	Claims Remaining After Amendment (after any cancellation)	Highest Number Previously Paid For	Extra Claims	Rate		Fee
Total Claims		-	= 0	x	\$18 =	\$0.00
Independent		-	= 0	x	\$80 =	\$0.00
Total of above calculations						\$0.00
<input type="checkbox"/> Reduction by 50% for filing by small entity						\$0.00
Total Additional Fee						\$0.00

- ☐ Please charge Deposit Account No. 50-1442 in the amount of _____ . A duplicate copy of this sheet is enclosed.
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10/10/00
Date


Signature of Applicant, Attorney or Agent of Record

STEVEN B. KELBER
REGISTRATION NO: 30,073

Typed or printed name

DOCKET NO. 9491-050-27 REISSUE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE REISSUE U.S. PATENT 4,968,603 ART UNIT:
APPLICATION OF:
SERIAL NO.: NEW REISSUE APPLICATION EXAMINER:
FILING DATE: HERewith
FOR: DETERMINATION OF STATUS IN NEOPLASTIC DISEASE

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, entry of the following amendments is respectfully requested.

IN THE CLAIMS:

Please amend the below listed claims:

17. (Amended) A method for determining the proper course of treatment for patients suffering from breast or ovarian adenocarcinoma, said method comprising:

determining the number of copies of the HER-2/neu gene in cells from a sample from a patient suffering from breast or ovarian adenocarcinoma;


identifying patients having at least two copies of the HER-2/neu gene in the cells, [which] and classifying those patients as patients who may require treatment proper for patients having a lesser chance of survival or decreased time to relapse; and

identifying patients having only a single copy of the HER-2/neu gene in the cells, [which] and classifying those patients as patients who may require treatment proper for patients having a greater chance of survival and being less likely to suffer disease relapse.

20. (Amended) A method as in claim 17, further comprising identifying those patients having five or more copies of the HER-2/neu gene, [which] and classifying those patients as patients who may require treatment proper for patients having a lesser chance of survival or decreased time to relapse due to metastasis.

Respectfully submitted,

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1. Field of the Invention

The ability to monitor neoplastic disease status can be of great value. In addition to improving prognostication, knowledge of the disease status allows the attending physician to select the most appropriate therapy for the individual patient. For example, patients with a high likelihood of relapse can be treated rigorously, usually involving systemic chemotherapy and/or radiation therapy. When there is a lesser likelihood of relapse, less aggressive therapies can be chosen. Because of the severe patient distress caused by the more aggressive therapy regimens, it would be desirable to distinguish with a high degree of certainty those patients requiring such aggressive therapies.

In the case of breast adenocarcinomas, the decision for chemotherapy after surgical removal of the tumor is usually based on the number of lymph nodes affected. A determination of nodal status, however, is still insufficient to confidently predict the status of the disease.

It would therefore be desirable to provide alternative factors which can be measured in order to determine the status of neoplastic diseases in human patients, particularly breast and ovarian adenocarcinomas. It would be further desirable if such factors provided a high correlation with disease status and allowed for determination of status at an early stage in the disease.

A human proto-oncogene appearing to have tyrosine kinase activity was independently identified by three research groups: Semba et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:6497-6501 (designating the gene *c-erbB-2*); Coussens et al. (1985) *Science* 230:1132-1139 (designating the gene *HER2*); and King et al. (1985) *Science* 229:974-976. Hereinafter, this gene is referred to as *HER-2/neu*. A related rat gene

(designated neu) was reported in Schecter et al. (1985) *Science* 229:976-978. Amplification of the gene and/or increased translation of expression of the gene have been observed in tumor cells and cell lines. See, e.g., Fukushima et al. (1986) *Mol. Cell. Biol.* 6:955-958 where amplification and elevated expression (mRNA) of the gene was observed in the MKN-7 gastric cancer cell line; Coussens et al. (1985) *supra*, where elevated transcription of the gene was observed in cell lines from a hepatoblastoma, a Ewing sarcoma, a rhabdomyosarcoma, two neuroblastomas, and a Wilms tumor; and King et al. (1985) *supra*, where amplification of the

gene was observed in a mammary carcinoma cell line. Yokota et al. (1986) *Lancet* I:765-767 disclose amplification of the HER-2/neu gene observed in breast, kidney, and stomach adenocarcinomas. Akiyama et al. (1986) *Science* 232:1644-1646 discloses the preparation of antibodies against a synthetic 14 amino acid fragment of the HER-2/neu gene product. The antibodies were used to isolate and characterize the gene product as it is expressed in MKN-7 adenocarcinoma cells.

SUMMARY OF THE INVENTION

The survival of a patient with a neoplastic disease, such as a breast or ovarian adenocarcinoma, can be determined by screening a patient sample for amplification of the HER-2/neu gene. Gene amplification has been found to correlate with both a decreased chance of long term survival as well as a shortened time to relapse of the disease. Patients displaying such gene amplification, even at relatively early stages of the disease, may be treated more rigorously in order to increase their chances for survival.

Gene amplification is defined to be at least two or more copies of the HER-2/neu gene, often being five or more copies of the gene, and frequently being as many as twenty or more copies of the gene. Gene amplification and/or expression may be measured in the patient sample either directly, for example, by Southern blotting (DNA analysis) using an appropriate probe, such as pCER 204, pMAC117, or λ 107. Gene expression, alternatively, may be measured either by Northern blotting to quantitate the transcription of mRNA, or by immunological methods, such as immunohistochemical staining of tissue sections and assay of body fluids, to directly quantitate the expression of gene product.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The HER-2/neu gene was independently isolated by several research groups and has been designated in various ways, including c-erbB-2 (Semba et al. (1985) *supra.*), HER-2 (Coussens et al. (1985) *supra.*), and MAC117 (King et al. (1985) *supra.*). The HER-2/neu gene encodes an approximately 185 kilodalton (kd) glycoprotein which appears to possess tyrosine kinase activity and is closely related to but distinct from the epidermal growth factor receptor (EGFR) gene. HER-2/neu is found on band q21 of chromosome 17 of the human genome and generates messenger RNA (mRNA) transcripts which are 4.8 kilobases (kb) in length. The nucleotide sequence of the gene suggests that the resulting protein may have an extracellular domain, a transmembrane domain that includes two cysteine-rich repeat clusters, and an intracellular kinase domain, indicating that it may be a cellular receptor for a ligand.

The present invention depends on the detection of amplification of the HER-2/neu gene as a measure of patient disease status and survivability. In particular, such gene amplification is directly related to the invasiveness of the disease and likelihood that the tumor has or will metastasize. Thus, patients who test positively for HER-2/neu gene amplification are less likely to survive and will usually suffer a shorter time to relapse after surgical removal of the tumor than patients without such amplification. Thus, the patients displaying gene amplification may benefit from aggressive treatment regimens after surgical removal of their tumors.

Conversely, patients who do not display gene amplification may be less likely to require such rigorous treatment.

The present invention is useful for screening a wide variety of neoplastic diseases, including both solid tumors and hemopoietic cancers. Exemplary neoplastic diseases include carcinomas, such as adenocarcinomas and melanomas; mesodermal tumors, such as neuroblastomas and retinoblastomas; sarcomas, such as osteosarcomas, Ewing's sarcoma, and various leukemias; and lymphomas. Of particular interest are adenocarcinomas of the breast, ovaries, colon, lung, stomach, and liver, more particularly of the breast and ovaries.

Depending on the nature of the cancer, an appropriate patient sample is obtained. In the case of solid tumors, a tissue sample from the surgically removed tumor will be obtained and prepared for testing by conventional techniques. In the case of lymphomas and leukemias, lymphocytes, leukemic cells, or lymph tissues will be obtained and appropriately prepared. Other patient samples, including urine, serum, sputum, cell extracts, etc. will also find use with particular tumors.

Once the patient tissue or cell sample is obtained, detection of gene amplification can be accomplished in a variety of ways. Conveniently, gene amplification may be measured directly by DNA analysis such as Southern blot or dot blot techniques. Such blotting techniques require the use of a labeled DNA probe, typically a radiolabelled probe specific for the HER-2/neu gene which is being measured. Suitable probes are described in the scientific literature; see for example, Semba et al. (1985) *supra*; King et al. (1985), *supra*., and Coussens et al. (1985), *supra*. Probes may also be prepared synthetically based on the known nucleotide sequences of the HER-2/neu gene reported in the same references.

For Southern blotting, high molecular weight DNA is obtained from the cells of the patient sample by conventional methods. The DNA is then digested with one or more restriction enzymes, and the resulting fragments separated on an agarose gel by electrophoresis. The DNA fragments are then transferred to a nylon or cellulose nitrate filter by blotting, and the DNA fixed by baking. The filter is then exposed to a labeled complementary probe and the regions of hybridization detected, usually by autoradiography. Dot blotting is similar, except that the DNA fragments are not separated on the gel. The degree of gene amplification is then determined by dilutional analysis or densitometry scanning, as described in the Experimental section hereinafter.

Alternately, gene expression (which corresponds to gene amplification) may be measured based on the level of mRNA transcription and/or gene product. mRNA transcription can be measured by a variety of techniques, including Northern blotting (Thomas (1980) Proc. Natl. Acad. Sci. USA 77:5201-5205), dot blots, and *in situ* hybridization. A variety of methods for measuring expression of the gene product exist, including Western blotting and immunohistochemical staining. Western blots are run by spreading a protein sample on a gel, usually an SDS gel, blotting the gel with a cellulose nitrate filter, and probing the filters with labeled antibodies. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzy-

matic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu et al. (1980) Am. J. Clin. Path. 75:734-738.

Gene amplification may also be determined indirectly by assay of a patient body fluid to detect the presence of elevated levels of the gene product. Suitable body fluids include serum, urine, and breast exudate, and a wide variety of useful immunoassays are described in the patent and scientific literature. See, for example, U.S. Pat. Nos. 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

Antibodies useful for immunohistochemical staining and/or assay of body fluids may be either monoclonal or polyclonal. Conveniently, the antibodies may be prepared against a synthetic peptide based on the reported DNA sequences of the HER-2/neu gene. Such synthetic peptides may then be used as an immunogen in preparing antibodies by well-known techniques. Alternatively, the natural gene product and/or portions thereof may be isolated and used as the immunogen.

Desirably, measurement of gene amplification will be performed quantitatively so that the number of gene copies can be estimated. Status of the disease correlates directly with the number of gene copies present in the tumor cells, and patients displaying even two copies of the HER-2/neu gene copies are at a higher risk of relapse than patients not displaying gene amplification. Moreover, as the number of HER-2/neu gene copies increases, the invasiveness and likelihood of metastasis also appears to increase. Thus, the number of gene copies should be taken as an important factor in assessing the disease status along with the traditional factors, including lymph node status, estrogen receptor status, progesterone receptor status, and the like. With all this information available, the treating physician is best able to assess the disease status and recommend the best treatment strategy to benefit the patient.

The following examples are offered by way of illustration, not by way of limitation.

EXPERIMENTAL

Tissue samples from 189 breast malignancies were obtained from an ongoing breast cancer study. Information available on the majority of the specimens included size of the primary tumor, estrogen receptor status, progesterone receptor status, disease stage, and status of the regional lymph nodes.

Initially, tissue samples from 103 primary breast cancers were evaluated for alternations in the HER-2/neu gene. DNA from individual tumors was prepared as described in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, pp. 282-285 (Cold Spring Harbor Laboratory, 1982), digested with EcoRI, and subjected to Southern blot analysis using a ³²P-labeled HER-2/neu-I probe, which is known to detect a 13 kb hybridizing band in human DNA (Coussens et al. (1985) Science 230:1132).

DNA was extracted from tissues and digested with EcoRI restriction endonuclease, as described (Maniatis et al., *Molecular Cloning: A Laboratory Manual*, pp. 282-285 (Cold Spring Harbor Laboratory), 1982). A total of 12 µg of EcoRI-digested DNA was loaded onto 0.8% agarose gels, electrophoresed, and transferred onto nylon filter papers (Biodyne) (Maniatis et al., supra.). All filters were baked in a vacuum oven for three

hours at 80° C., pre-hybridized in 5X SSC (1X SSC is 0.15M NaCl/0.015M sodium citrate, pH 7.0) containing 50% formamide, 10% dextran sulfate, 0.1% SDS, 1 mg/ml of denatured salmon sperm DNA, and 4X Denhardtts (100X Denhardtts being 2% BSA, 2% ficoll, and 2% polyvinyl pyrrolidone) for twelve hours, then hybridized in the same solution containing 2×10^6 cpm/ml of nick-translated HER-2 probe (Schechter et al., (1984) Nature 312:5113) which had been labeled with ^{32}P to a specific activity of 1×10^8 cpm/ μg DNA. Hybridizing occurred at 42° C. for 48 hours, followed by washing of filters in 2X SSC for twenty minutes at room temperature, followed by two successive washes of thirty minutes each in 2X SSC, 0.1% SDS at 65° C., followed by one wash of thirty minutes in 0.5X SSC, 0.1% SDS at 65° C. Filters were then exposed to XAR-5 X-ray film (Kodak, Rochester, N.Y.) for autoradiography.

Dilutional analysis was then performed to assess the degree of HER-2/neu gene amplification. DNA was diluted in water or buffer to various dilutions, such as 1:2, 1:5, 1:10, 1:20, 1:100, etc., and Southern analysis performed along side single copy DNA samples for comparison.

Of the 103 samples examined, nineteen (18%) showed evidence of HER-2/neu gene amplification. The degree of amplification in individual cases was determined by dilutional analysis, as well as soft laser densitometry scanning. To determine that the amount of DNA loaded in each lane was equivalent, all filters were washed and rehybridized with a ^{32}P -labeled arginase gene probe. This probe identifies a 15 kb hybridizing band on EcoRI-digested human DNA, and was selected as a control because it more appropriately assesses the relative amount and transfer of high molecular weight species than a probe hybridizing with low molecular species which transfer more readily on Southern blotting. All lanes were shown to contain equivalent amounts of high molecular weight DNA. Individual tumors were assigned to groups containing single copy, two to five copies, five to twenty copies, and greater than twenty copies of the HER-2/neu gene. Assignment of tumors to the various groups was done in a blinded fashion, in that they were made without knowledge of disease parameters. Analysis of the data for correlation between gene amplification and a number of disease parameters was then performed.

One hundred and three tumors were evaluated in the initial survey. There was no correlation between gene amplification and estrogen receptor status, with 7/38 (18%) of patients with amplification having less than 3 femtomoles/mg protein, and 12/65 (18%) having more than 3 femtomoles (p value=0.99); no correlation with progesterone receptor status, with 9/51 (18%) having less than 5 femtomoles, and 10/52 (19%) having more than 5 femtomoles (p value =0.85); no correlation with size of tumor, with 2/15 (13%) of patients having tumors less than 2.5 cm. 7/41 (17%) having tumors 2.5-5 cm, and 5/22 (23%) having tumors more than 5 cm in size (p value =0.82); and no correlation with age at diagnosis, with 4/25 (26%) being less than 50 years old and 13/65 (20%) being more than 50 years old (p value =0.99)(Table 1).

When analysis was performed for correlation between HER-2/neu amplification and number of positive lymph nodes, however, a trend was noted. Gene amplification was found in 4 of 34 patients (11%) with no involved nodes, 2 of 20 patients (10%) with one to three involved nodes, and 8 of 25 patients (32%) with more

than three involved nodes (p value = 0.11). If these data were examined comparing 0-3 positive nodes versus greater than 3 positive nodes, the correlation with number of nodes became more significant (p value ≤ 0.03) (Table 1). Thus, there was a significant increase in incidence of HER-2/neu gene amplification in patients with more than three axillary lymph nodes involved with disease. A multivariate regression analysis to correlate HER-2/neu amplification with various disease parameters identified the number of positive nodes as the only significant factor, either alone or in combination, to correlate with gene amplification.

TABLE I

Correlation of HER-2/neu Amplification With Various Disease Parameters in 103 Breast Tumors					
	Single Copy	2-5 copies	5-20 copies	>20 copies	To- tal P-value**
Estrogen Receptor Status					
ER+	53	2	9	1	65 p = 0.99
ER-	31	1	2	4	38
Progesterone Receptor Status					
PgR+	42	2	6	2	52 p = 0.85
PgR-	42	1	5	3	51
Tumor Size					
<2.5 cm	13	1	1	0	15 p = 0.82
2.5-5 cm	34	1	5	1	41
>5 cm	17	1	2	2	22
UNK*	20	0	3	2	25
Patient Age					
<50 years	21	1	2	1	25 p = 0.83
>50 years	52	2	7	4	65
UNK*	11	0	2	0	13
Number of Involved Nodes					
0 nodes	30	0	3	1	34 p = 0.11
1-3 nodes	20	0	1	1	22 p ≤ 0.05
>3 nodes	17	2	4	2	25 for 0-3
UNK*	17	1	3	1	22 vs >3
Total	84	3	11	5	103

*UNK = unknown.

**P-values arrived at by combining 5-20 and >20, since there were so few samples in the >20 group. Statistical analyses for correlation of HER-2/neu amplification with various disease parameters were performed by the chi-square test.

Further study was conducted on 100 breast cancer tissue samples from patients with positive axillary lymph nodes. All of the information available for the first group of 103 patients was also available for these patients. In addition, relapse and survival information was available, since these cases had a median follow-up of 46 months (range 24-86 months). Of these 100 samples, 86 yielded sufficient DNA for study. Amplification of the HER-2/neu gene was measured as in the initial survey, and amplification was found in 34 of the 86 patients (40%). The parameters which were analysed for correlation to HER-2/neu amplification were, again, estrogen receptor status (positive versus negative), progesterone receptor status (positive versus negative), size of tumor (less than 2.5 cm versus 2.5-5 cm versus more than 5 cm), age at diagnosis (less than 50 versus more than 50), and number of axillary nodes positive for disease involvement (1-3 versus more than 3). For this larger sample of node-positive patients, several statistically significant or nearly significant correlations were observed. In agreement with the preliminary survey, there was a correlation between number of involved lymph nodes and HER-2/neu amplification (Table II). In addition, there was a significant correlation with estrogen receptor status and size of primary

[illegible]

TABLE II

	Single Copy	2-5 copies	5-20 copies	>20 copies	Total	P-value**
Estrogen Receptor Status						
ER+	38	21	5	1	65	p = 0.05
ER-	14	2	4	1	21	
Progesterone Receptor Status						
PgR+	31	18	4	1	54	p = 0.14
PgR-	21	5	5	1	32	
Tumor Size						
<2 cm	18	8	3	0	29	p = 0.09
2-5 cm	28	12	2	1	43	p = 0.02
UNK*	6	3	4	1	14	for <5 vs >5
Patient Age						
<50 years	16	12	6	1	35	p = 0.06
>50 years	36	11	3	1	51	
Number of Involved Nodes						
1-3 nodes	31	7	5	0	43	p = 0.06
>3 nodes	21	16	4	2	43	
Total	52	23	9	2	86	

*UNK = unknown.

*P-values arrived at by combining 5-20 and >20, since there were so few samples in the >20 group. Statistical analyses for correlation of HER-2/neu amplification with various disease parameters were performed by the chi-square test.

TABLE III

Correlation of HER-2/neu Amplification With Various Disease Parameters in Combined Surveys (189 Patients)						
	Negative	2-5	5-20	>20	Total	P-value**
Estrogen Receptor Status						
ER+	91	23	14	2	130	p = 0.05
ER-	45	3	6	5	59	
Progesterone Receptor Status						
PgR+	73	20	10	3	106	p = 0.06
PgR-	63	6	10	4	83	
Tumor Size						
<2 cm	31	9	4	0	44	p = 0.19
2-5 cm	62	13	7	2	84	
>5 cm	23	4	6	3	36	
UNK*	20	0	3	2	25	
Patient Age						
<50 years	37	13	8	2	60	p = 0.11
>50 years	88	13	10	5	116	
UNK*	11	0	2	0	13	
Number of Involved Nodes						
0 nodes	30	0	3	1	34	p = 0.002
1-3 nodes	51	7	6	1	65	
>3 nodes	38	18	8	4	68	
UNK*	17	1	2	1	22	

*UNK = unknown.

*P-values arrived at by combining 5-20 and >20, since there were so few samples in the >20 group. Statistical analyses for correlation of HER-2/neu amplification with various disease parameters were performed by the chi-square test.

While these correlations were of interest, the strong association between HER-2/neu amplification and nodal status (p value=0.002) indicated that information on amplification of this gene may correlate with disease behavior, i.e., recurrences and survival. To test this, univariate survival analyses were performed comparing amplification to relapse and survival in this group of

patients. A strong and highly statistically significant correlation was found between gene amplification and both time to disease relapse (p value = <0.0001) and survival (p value=0.0011) (Table IV). Moreover, when compared to other parameters, amplification of HER-2/neu was found to be superior to all other prognostic factors, with the exception of the number of positive nodes (which it equaled) in predicting time to relapse and overall survival in human breast cancer (Table IV A).

TABLE IV A

Results of Univariate Survival Analyses In Node-Positive Patients ¹ Comparing Disease-Free Survival (Relapse) and Overall Survival to Factors Listed		
Factor	P-value Survival	P-value Relapse
Number of positive nodes	0.0001	0.0002
HER-2	0.0011	<0.0001
Log (PgR)	0.05	0.05
Size of Tumor	0.06	0.06
Log (ER)	0.15	0.10
Age	0.22	0.61

¹Patient cohort for both Table IV A and IV B consisted of 86 patients with node-positive breast cancer who had a median follow-up of 47 months (range 24-86 months). Median time to recurrence in this group was 62 months. Median time to death was 69 months.

TABLE IV B

Results of Multivariate Survival Analyses ² In Node-Positive Patients to Evaluate Predictive Value of Various Prognostic Factors in a Multivariate Manner		
SURVIVAL	Regression Coefficients (Standard Errors)	P-Value
Number of positive nodes	0.0938 ± 0.0256	p = 0.0003
HER-2	0.0872 ± 0.0388	p = 0.02
Log (ER)	-0.5158 ± 0.2414	p = 0.03
RELAPSE		
Number of positive nodes	0.0849 ± 0.0266	p = 0.001
HER-2	0.1378 ± 0.0425	p = 0.001

²Cox's partially non-parametric regression model was used to evaluate the predictive power of various combinations and interactions of prognostic factors in a multivariate manner.

To determine if amplification of HER-2/neu was an independent prognosticator of disease behavior, multivariate survival analyses were performed on the 86 node-positive cases, and again, amplification of the gene proved to be a strong prognostic factor (Table IV B). Rearrangement of the HER-2/neu gene was rare. Of the total 198 tumors evaluated, three showed evidence of rearrangement, and in two of the three cases, the rearrangement was identical. Also, two of the rearranged HER-2/neu loci were amplified. The incidence of HER-2/neu rearrangement as determined by EcoRI digestion was too small to allow statistical correlations.

To determine whether the phenomenon of amplification of HER-2/neu in breast cancer extended to related growth factor receptors, all filters were analysed with the EGFR probe (Ullrich et al. (1984) Nature 309:418). Amplification of the EGFR gene was found in 4/189 (2%) of the cases, and rearrangement of the EGFR gene was found in one of those four cases. The incidence of EGFR amplification and rearrangement was too small to allow statistical correlation. Comparison of HER-2/neu amplification (53/189 or 28%) with that of the EGFR gene reveals the incidence of the former to be fourteen times greater than that of the latter, indicating that the phenomenon of gene amplification is not a general one for a related tyrosine-kinase-specific receptor in human breast cancer.

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Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims and that use of this invention can extend to the detection, monitoring, and prognosis of a wide variety of human malignancies.

What is claimed is:

1. A method for screening patients to determine disease status, said method comprising:
measuring the level of amplification or expression of the HER-2/neu gene in a sample from a patient suffering from breast or ovarian adenocarcinoma;
and
classifying those patients having an increased level of amplification or expression of the HER-2/neu gene relative to a reference level characteristic of normal cells as being more likely to suffer disease relapse or having a decreased chance of survival.
2. A method as in claim 1, wherein gene amplification is measured directly by DNA analysis with a probe specific for the HER-2/neu gene.
3. A method as in claim 1, wherein gene expression is measured by determination of gene product.
4. A method as in claim 1, wherein gene expression is measured by determination of mRNA transcription.
5. A method as in claim 1, wherein the expression of the HER-2/neu gene is determined by measuring the amount of gene product using a liquid phase immunoassay.
6. A method as in claim 2, wherein the amplification of the HER-2/neu gene is determined indirectly by assay of a body fluid from a patient for increased levels of HER-2/neu expression.
7. A method for determining a prognosis in patients suffering from breast or ovarian adenocarcinoma, said method comprising:
determining the number of copies of the HER-2/neu gene in cells from a sample from a patient suffering from breast or ovarian adenocarcinoma; and
classifying patients having only a single copy of the HER-2/neu gene as being less likely to suffer disease relapse than those patients having two or more copies of the HER-2-neu gene.
8. A method as in claim 7, wherein patients having at least five copies of the HER-2/neu gene are classified as having an increased risk of metastasis compared to patients having fewer copies of the gene.
9. A method as in claim 7, wherein the number of copies of the HER-2/neu gene is determined directly by Southern or dot blotting.
10. A method as in claim 7, wherein the expression of the HER-2/neu gene is determined by measuring the amount of the HER-2/neu mRNA transcripts or gene product, said expression corresponding to gene amplification.
11. A method as in claim 10, wherein the expression of the HER-2/neu gene is determined by measuring the

amount of gene product by histochemical staining with labeled antibody specific for the gene product.

12. A method as in claim 10, wherein the expression of the HER-2/neu gene is determined by measuring the amount of gene product using a liquid phase immunoassay.

13. A method as in claim 7, wherein patients having more than two copies of the HER-2/neu gene are classified as having an increased risk of disease relapse compared to patients having only two copies of the gene.

14. A method as in claim 7, wherein the number of copies of the HER-2/neu gene is determined prior to lymph node involvement.

15. A method as in claim 7, wherein the number of copies of the HER-2/neu gene is determined indirectly by assay of a body fluid from a patient suffering from breast or ovarian adenocarcinoma for increased levels of HER-2/neu gene expression.

16. A method as in claim 15, wherein the fluid from a patient suffering from breast or ovarian adenocarcinoma is selected from the group consisting of serum, urine, and breast exudate.

17. A method for determining the proper course of treatment for patients suffering from breast or ovarian adenocarcinoma, said method comprising:

determining the number of copies of the HER-2/neu gene in cells from a sample from a patient suffering from breast or ovarian adenocarcinoma;

identifying patients having at least two copies of the HER-2/neu gene in the cells, which patients may require treatment proper for patients having a lesser chance of survival or decreased time to relapse; and

identifying patients having only a single copy of the HER-2/neu gene in the cells, which patients may require treatment proper for patients having a greater chance of survival and being less likely to suffer disease relapse.

18. A method as in claim 17, wherein the number of copies of the HER-2/neu gene is determined directly by Southern or dot blotting.

19. A method as in claim 17, wherein the expression of the HER-2/neu gene is determined by measuring the amount of HER-2/neu mRNA transcripts or gene product, said expression corresponding to gene amplification.

20. A method as in claim 17, further comprising identifying those patients having five or more copies of the HER-2/neu gene, which patients may require treatment proper for patients having a lesser chance of survival or decreased time to relapse due to metastasis.

21. A method as in claim 17, wherein the proper treatment includes systemic chemotherapy or radiation therapy.

22. A method as in claim 17, wherein the number of copies of the HER-2/neu gene is determined prior to lymph node involvement.

* * * * *

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--23. A method for screening patients to determine disease status, said method comprising:

measuring the level of amplification or expression of the HER-2/neu gene in a sample from a patient suffering from breast or ovarian adenocarcinoma;

identifying the number of lymph nodes involved in said patient;

correlating amplification or expression of the HER-2/neu gene in the sample to having three or more involved lymph nodes; and

classifying those patients having (1) an increased level of amplification or expression of the HER-2/neu gene relative to a reference level characteristic of normal cells; and (2) having three or more involved lymph nodes as being more likely to suffer disease relapse or having a decreased chance of survival.

24. A method for determining a prognosis in patients suffering from breast or ovarian adenocarcinoma, said method comprising:

determining the number of copies of the HER-2/neu gene in cells from a sample from a patient suffering from breast or ovarian adenocarcinoma;

identifying the number of lymph nodes involved in said patient;

correlating having two or more copies of the HER-2/neu gene in the sample to having three or more involved lymph nodes; and

classifying (a) patients having (1) only a single copy of the HER-2/neu gene; and (2) less than three involved lymph nodes as being less likely to suffer disease relapse than (b) those patients having (1) two or more copies of the HER-2-neu gene; and (2) three or more involved lymph nodes.

25. A method for determining the proper course of treatment for patients suffering from breast or ovarian adenocarcinoma, said method comprising:

[11] Patent Number: 4,968,603
[45] Date of Patent: Nov. 6, 1990

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Primary Examiner—Robert A. Wax
Assistant Examiner—Stephanie W. Zitomer
Attorney, Agent, or Firm—Townsend and Townsend

[57] ABSTRACT

Amplification of the HER-2/neu oncogene is related to the status of neoplastic diseases, particularly breast and ovarian adenocarcinomas. The presence of multiple gene copies in tumor cells indicates that the disease is more likely to spread beyond the primary tumor site, and that the disease therefore may require more aggressive treatment than might otherwise be indicated by other diagnostic factors. In particular, the degree of gene amplification appears to provide greater prognostic utility than either the estrogen receptor or the progesterone receptor, and provides utility equal to that of the determination of lymph node status. The information provided by the gene amplification test, however, is not duplicative with the determination of lymph node status and the two tests together provide greatly improved prognostic utility.

22 Claims, No Drawings

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DOCKET NO. 9491-050-27 REISSUE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE REISSUE U.S. PATENT 4,968,603 ART UNIT:
APPLICATION OF:
SERIAL NO.: NEW REISSUE APPLICATION EXAMINER:
FILING DATE: HERewith
FOR: DETERMINATION OF STATUS IN NEOPLASTIC DISEASE

REISSUE DECLARATION

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

We, Regents of the University of California; The Board of Regents for the University of Texas System and Genentech, Inc., do hereby declare and state that:

1. We are the joint assignees of U.S. Patent 4,968,603, and the reissue application therefor submitted herewith.
2. All of us are legal persons of the United States of America.
3. We have reviewed and understand the contents of the Specification, including the claims, of the reissue application for U.S. Patent 4,968,603 (reissue application) submitted herewith.
4. We believe Dennis J. Slamon, William L. McGuire and Axel Ullrich to be the original and first inventors of the subject matter which is claimed in the reissue application for which a reissue patent is sought.
5. We acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to patentability as defined in 37 C.F.R. §1.56, specifically, we are aware of our duty to disclose to the United States Patent and Trademark

Office all information known to us that establishes, by itself or in combination with other information, a case of unpatentability of a claim presented in the above-captioned application, without more, or refutes or is inconsistent with a position taken by us or on our behalf in either opposing an argument of unpatentability, or asserting an argument of unpatentability.

6. We claim the benefit of priority of U.S. Patent 4,968,603, and any claims to priority therein.

7. We believe U.S. Patent 4,968,603 to be wholly or partly inoperative or invalid by reason of our claiming less than we had a right to claim, as well as failing to claim specific embodiments that we had a right to claim. Specifically, we identify as an error, the failure to advance claims narrower than those which issued which recite identification of a patient suffering from breast or ovarian adenocarcinoma as well as amplification of the HER-21/neu as an indication of disease prognosis.

8. All errors being corrected by the reason of the filing of reissue application up to the filing of this Declaration arose without any deceptive intention on our part.

All statements made herein of our own knowledge are true and all statements made on information and belief are believed true. We are aware that willful false statements and the like are punishable by fine, imprisonment, or both, 18 U.S.C. §1001, and that such willful false statements may jeopardize the above-captioned reissue application, the validity of any patent to issue thereon, and the validity of U.S. Patent 4,968,603.

Regents of the University of California

Dated: _____

BY: _____

Title: _____

The Board of Regents for the University of Texas System

Dated: _____

BY: _____

Title: _____

Genentech, Inc.

Dated: _____

BY: _____

Title: _____

DOCKET NO. 9491-050-27 REISSUE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE REISSUE U.S. PATENT 4,968,603 ART UNIT:
APPLICATION OF:
SERIAL NO.: NEW REISSUE APPLICATION EXAMINER:
FILING DATE: HERewith
FOR: DETERMINATION OF STATUS IN NEOPLASTIC DISEASE

ASSENT OF ASSIGNEE

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

The (1) Regents of the University of California of Berkeley, California, (2) The Board of Regents for the University of Texas System of San Antonio, Texas, and (3) Genentech, Inc. of south San Francisco, California, are the owners of the entire right, title and interest in and to U.S. Patent 4,968,603, by reason of the assignments of all inventors interests therein, which assignments have been registered at the United States Patent and Trademark Office (e.g., Reel 4707/Frame 0977 (Regents of the University of California); Reel 5637/Frame 0484 (Board of Regents, the University of Texas System) and Reel 5594/Frame 0297 (Genentech, Inc.). On behalf of Regents of the University of California; The Board of Regents for the University of Texas System and Genentech, Inc., I hereby state that the assignments of the inventors in favor of Regents of the University of California; the Board of Regents for the University of Texas System and Genentech, Inc. of U.S. Patent 4,968,603 have been reviewed and, to the best of my knowledge and belief, title is in Regents of the University of California; The Board of Regents for the University of Texas System and Genentech, Inc.

Regents of the University of California; The Board of Regents for the University of Texas

System and Genentech, Inc hereby assent to the filing and prosecution of a reissue application seeking reissue of U.S. Patent 4,968,603, filed herewith, pursuant to the reissue declaration of the Assignees submitted herewith.

Regents of the University of California

Dated:_____

BY:_____

Title:_____

The Board of Regents for the University of Texas System

Dated:_____

BY:_____

Title:_____

Genentech, Inc.

Dated:_____

BY:_____

Title:_____

DOCKET NO. 9491-050-27 REISSUE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE REISSUE U.S. PATENT 4,968,603 ART UNIT:
APPLICATION OF:
SERIAL NO.: NEW REISSUE APPLICATION EXAMINER:
FILING DATE: HERewith
FOR: DETERMINATION OF STATUS IN NEOPLASTIC DISEASE

OFFER OF SURRENDER, 37 C.F.R. §1.171

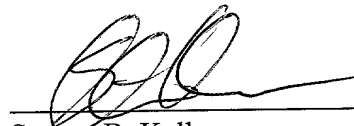
ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Reissue Applicants, through undersigned counsel, hereby offer to surrender the original patent, U.S. Patent 4,968,603 upon allowance of the case.

Respectfully submitted,

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United States Patent [19]
Slamon et al.

[54] **DETERMINATION OF STATUS IN
NEOPLASTIC DISEASE**

[75] **Inventors:** Dennis J. Slamon, Woodland Hills,
Calif.; William L. McGuire, San
Antonio, Tex.

[73] **Assignee:** The Regents of the University of
California, Berkeley, Calif.

[21] **Appl. No.:** 948,265

[22] **Filed:** Dec. 31, 1986

[51] **Int. Cl.⁵** C12Q 1/68; G01N 53/00;
G01N 33/00

[52] **U.S. Cl.** 435/6; 435/7;
935/77; 935/78; 436/501; 436/94

[58] **Field of Search** 435/6, 7; 935/77, 78;
436/501, 94

[56] **References Cited**

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